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(54) Title: PRE-SCREENING FOR DEPLETION AND ENRICHMENT OF SPECIFIC B-CELLS

(57) Abstract

Disclosed is a pre-screening process wherein antigens are used to select for certain B-cells which are found in a family of B-cells. In one embodiment, reactive B-cells are bound to an antigen and then the non-reactive B-cells in the supernatant are collected, (known as depletion). Alternatively, the B-cells which one wishes to select can be bound directly, (known as enrichment). In another embodiment, B-cells to a first antigen with a specific epitope are selected in suspension. This is done by adding a second antigen with at least one epitope in common with the first antigen to a suspension of B-cells and forming aggregates of B-cells which produce antibody to the second antigen. The supernatant, which wll contain B-cells producing antibody to the specific epitope, is then collected. In yet another embodiment, B-cells carrying anti-idiotype antibody are selected by locating aggregates in a B-cell suspension. The aggregates are made up of anti-idiotype carrying B-cells and other B-cells which carry antibody. The B-cells selected by any of the above methods can be fused with myeloma cells and used to produced monoclonal antibodies.

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"PRE-SCREENING FOR DEPLETION AND ENRICHMENT OF SPECIFIC B-CELLS"

Field of the Invention

The invention relates to the field of immunology, and more particularly to a pre-screening procedure for selecting B-cells producing antibodies to specific epitopes carried on antigens. The selected B-cells can be used for production of monoclonal antibodies.

Background of the Invention

B-cell lymphocytes are the cells of an animal which produce antibody when the animal is exposed to an antigen. When an antigen enters the body it causes a complex series of responses in the immune system. Certain B-cells are stimulated to produce antibody which can bind to the epitopes of that antigen. Some of the antibody produced is excreted by the B-cell, while some is carried on the cell surface.

B-cells have become of particular interest to the scientific community in recent years because they are essential in the production of monoclonal antibodies. Monoclonal antibodies have a wide variety of applications. Their primary use is in detecting the presence of particular proteins or other antigens. Detection of such molecules can be valuable for diagnosis of disease states or diagnosis of other conditions, such as pregnancy. Monoclonal antibodies are also useful in research, in collecting and purifying specific antigens, and in the development of vaccines.

The properties of monoclonal antibodies makes them suited for these applications. Monoclonal antibodies bind specifically to a particular epitope of an antigen.

They have this property because they are produced by cells, known as hybridomas, which are all cloned from a single cell. All the clones have the same genetic pattern as the parent. Accordingly, all clones produce identical antibodies which have identical characteristics.

A method of making monoclonal antibodies was first described by Koehler and Milstein. See Milstein et al., Nature, 256, 495-97 (1975); Koehler et al., Eur. J. Immunol., 6, 511-19 (1976). A host animal, usually a mouse, is immunized with an antigen and then sacrificed. The tissue which is rich in B-cells, usually the spleen or lymphoid tissue, is ground to expose the B-cells. The ground tissue is fused with myeloma (cancer) cells to form hybridomas. The hybridomas which produce antibody to the epitopes of the immunizing antigen are then isolated from the others, many of which produce antibodies to other antigens. The selected hybridomas are then reproduced to manufacture monoclonal antibodies.

The most difficult step in the monoclonal antibody production procedure is isolating the hybridomas desired, i.e., those which produce antibody to the epitopes of the immunizing antigen. Hybridomas which produce undesired antibody, or no antibody at all, are formed when B-cells which respectively produce undesired antibody, or no antibody, are fused with myeloma cells. B-cells which produce undesired antibody are present because every animal is exposed to numerous antigens during its lifetime, and each antigenic exposure stimulates B-cells to produce reactive antibody. B-cells which produce no antibody are those which are never stimulated by antigens during the animal's lifetime.

Some of both types of B-cells are always present in a tissue sample.

The desired hybridomas are typically isolated by sufficiently diluting the hybridoma cell suspension which results from the fusion to ensure the presence of only one clone in a given volume of suspension. The volume of the suspension which contains the single clone is then placed into a well. The antibody produced by the hybridoma, which is excreted by the clone and which therefore becomes suspended in the well, is then bound to the walls of the well. Thereafter, radioactively labeled immunizing antigen is placed into each well. If the antibody in the well is specific to the labeled antigen, the labeled antigen will be bound. If the antibody in the well is not specific to the labeled antigen, i.e., the antibody is undesired, the labeled antigen will remain suspended. The wells are then washed to remove any unbound labeled antigen. Clones from those wells which display the highest level of radioactivity, and thus the highest production of the desired antibody, are multiplied to produce desired monoclonal antibodies.

If the B-cells which produce antibody to the immunizing antigen could be separated from those which produce no antibody, this would shorten the isolation process which is used to locate desired hybridomas as described above. If a pre-screen selection of B-cells were performed, non-producing B-cells would not be used in the fusion, and fewer of the hybridomas would be undesired. A larger proportion of wells would contain desired hybridomas. Isolation of desired hybridomas would consequently be faster and easier.

A B-cell pre-screening procedure can be utilized in many specific applications. One application is when the objective is to produce monoclonal antibodies specific to only one of the multiple epitopes that are present on an antigen. The isolation process where radioactively labeled antigen is used to locate hybridomas as described above, would not allow selection of hybridomas which produce antibody only to the designated epitope. The aforementioned isolation process relies on selective binding of radioactively labeled antigen as a means for identifying appropriate antibodies. Antibodies which are to any of the many epitopes of the labeled antigen -- whether it is the designated epitope or not -- will bind the labeled antigen. Thus, wells which contain antibodies to the undesired epitopes on the antigen will display a high radioactivity level. In fact, wells containing antibodies to undesired epitopes will bind labeled antigen to the same extent and display as high a radioactivity count as wells which contain antibody to the designated epitope.

At present, the selection of hybridomas producing monoclonal antibodies which are specific to one of the epitopes on an antigen is done by breaking the antigen into subunits, if the particular antigen can be broken down. The monoclonals are then tested for reactivity with the various subunits and with the whole antigen. The monoclonals which react only with the whole antigen, and not with the subunits, are then isolated.

The problem with this procedure is that only a small proportion of the desired hybridomas are present, and the selection for them is thus extremely lengthy. If B-cells could be pre-screened prior to fusion to select those which produce antibody to only the desired

epitope, a greater proportion of desired hybridomas would result from the fusion of the pre-screened B-cells. It would thus be easier to isolate the desired hybridomas when using the hybridoma selection process that is described above.

Another application of a B-cell pre-screening procedure is where different immunogenic entities carry some of the same epitopes, and the objective is to produce monoclonal antibodies to the epitopes which are not common to all entities. An example of such immunogenic entities is a normal cell and a cancerous cell. Both will have a large number of surface epitopes in common. Yet the cancerous cell should carry some epitopes which represent the alteration in its structure.

It would be extremely time-consuming to isolate hybridomas which produce monoclonals to the unique epitopes of the cancer cell. The monoclonals would have to be produced, and then those from different hybridomas would have to be tested for reactivity with both normal and cancerous cells. Due to the wide variety of epitopes on the surface of cells, the vast majority of the monoclonals which react with cancerous cells would also react with normal cells. Isolating monoclonals which react only with cancerous cells would be like looking for a needle in a haystack.

If the B-cells were pre-screened to select for those which produce antibody to the unique epitopes on the cancerous cells, this would result in a greater proportion of hybridomas which produce monoclonals to the unique epitopes. Due to the presence of a greater proportion of desired hybridomas, the time needed to

isolate the desired hybridomas from others would be reduced.

A B-cell pre-screening process can also be used to isolate antibodies to uncommon epitopes on other types of cells. For example, a B-cell pre-screening process could reduce the time involved with isolating antibodies which differentiate diseased cells or virus transformed cells from normal cells. In addition, a pre-screening process could reduce the time involved with differentiating antibodies to young cells from those to quiescent cells or senescent cells, or differentiating antibodies to starved cancer cells from those to non-starved cancer cells, or differentiating antibodies to transformed cells causing arteriosclerosis from those to normal epithelial cells which line the blood vessels.

For the same reasons that it is advantageous in reducing the production time in the above situations, a B-cell pre-screening process would also be useful for reducing the time needed to produce antibodies to certain biological molecules which are closely related, or even nearly identical, to other biological molecules. For example, the time involved with producing antibodies to different strains of viruses, and to fully folded, slightly folded, and unfolded proteins could be reduced.

A B-cell pre-screening process could be useful in isolating B-cells producing antibodies which are specific to certain trace proteins, such as those associated with disease states. If th protein is truly a trace protein, by definition present only in minute quantities, a correspondingly small number of B-cells will produce antibody to it. If hybridomas are produced from the family of B-cells, then the few hybridomas which result from the fusion of these few B-cells will

be very difficult to isolate from those surrounding them. However, if the B-cells were pre-screened to select those producing antibody to the trace protein, a greater proportion of the hybridomas in the wells would produce monoclonals to the trace proteins. Therefore, isolation of the desired hybridomas would be easier.

A B-cell pre-screening process could also be useful in isolating anti-idiotype antibodies. All antibodies carry "idiotypes," regions near their antigen-recognition sites that are themselves capable of acting as antigens and stimulating antibody production. See J.L. Marx, Science, 228 "Making Antibodies Without Antigens," 162-65 (1986). Antibodies which are specific to the idiotype sites are known as anti-idiotype antibodies. Anti-idiotype antibodies have the same molecular structure, at least in part, as does the antigen which stimulated antibody production.

Anti-idiotype antibodies can be made by immunizing an animal with antibody. In order to make the antibody, a particular antigen is first selected, and an animal is immunized with it. If a pathogenic antigen is selected, the anti-idiotype antibodies eventually produced will be suitable for use as vaccines. The anti-idiotype will be so suited because they will carry the same molecular structure, at lease in part, as the antigen. Thus, immunization of a healthy individual with anti-idiotype antibodies will cause the same immune response as does immunization with the pathogenic antigen. The anti-idiotype antibody will, therefore, immunize the individual to the pathogen. However, the anti-idiotypes are themselves totally non-pathogenic.

It would be extremely valuable, given the potential for use as vaccines, to produce large quantities of

monoclonal anti-idiotype antibodies. The difficulty with making such monoclonals is that anti-idiotype antibodies are extremely rare and difficult to isolate. As a result, the normal post-fusion isolation process is lengthy and uncertain. A pre-screening process for increasing the proportion of anti-idiotype producing B-cells would increase the likelihood of producing a large proportion of desired hybridomas.

In one embodiment of the present invention, a B-cell pre-screening procedure is done by binding an antigen, or cell, to a solid support, and passing a family of B-cells over the antigen. The B-cells producing antibody specific to the bound antigen carry that antibody on the cell surface. As a result these B-cells bind to the antigen.

An alternate type of pre-screening is done completely in suspension. This type of pre-screening is particularly useful when attempting to isolate anti-idiotype antibodies. B-cells producing anti-idiotype antibody and carrying it on their surface, will tend to aggregate with B-cells which produce and carry the idiotype (antibody). Accordingly, in this type of pre-screening one locates aggregates in suspension and then examines the aggregates for the presence of anti-idiotypes.

Alternatively, a B-cell pre-screening in suspension could be used to isolate specific B-cells. B-cells to a specific epitope on a first antigen are selected in suspension by eliminating the B-cells to the other epitopes on that antigen. This is done by aggregating the B-cells carrying antibody to the other epitopes. The supernatant, which will contain a greater proportion of desired B-cells, is then collected.

A pre-screening process where an antigen is used to selectively bind certain B-cells, used as part of a monoclonal antibody production procedure, has never been done. Although solid support selection processes have been used in the past, these involved antibodies, not antigen, bound to the solid support. Such processes are disclosed in the following articles: M.G. Mage et al., "Mouse lymphocytes with and without surface immunoglobulin: Preparative scale separation on polystryrene tissue culture dishes coated with specifically purified anti-immunoglobulin." J. Immunol. Methods, 15:47-56 (1977); D.L. Williams, et al. "Enrichment of T Lymphocytes from Bovine Peripheral Blood Mononuclear Cells Using an Immuno-Affinity Depletion Technique ("Panning"), Vet. Immunology and Immunopathy, 11:199-204 (1986). Nevertheless, one study does show binding between cells bound to a plate and other cells. Gordon et al. incubated Human Blast Progenitors with stromal cells which had been grown on plates, in order to separate the former from other colony-forming cells. <u>See</u> M.Y. Gordon, et al., "Separation of Human Blast Progenitors from Granulocytic, Erythroid, Megakaryocytic, and Mixed Colony-forming Cells by "Panning" on Cultured Marrow-derived Stromal Layers," Experimental Hematology, 13:937-940 (1985). In this study, however, B-cells were not being selected.

No one has ever disclosed or suggested using a B-cell pre-screening process, which involved locating aggregates, as a way of isolating anti-idiotype antibodies. The connection between aggregates and the presence of anti-idiotypes simply has not been appreciated. No one has ever disclosed or suggested carrying out a B-cell pre-screening in suspension.

The advantages of the invention will become apparent from the following description.

Summary of the Invention

The invention relates to a B-cell pre-screening process where antigens are used to select certain B-cells from a family of B-cells. Hereinafter, the term "antigen" includes cells, proteins or any other antigens. In one embodiment, a family of B-cells which have been taken from an immunized animal is exposed to antigen bound to a solid support. The antigen chosen is one which selectively binds certain of the B-cells. Following the pre-screening, the selected B-cells are fused to produce hybridomas, which in turn yield monoclonal antibodies.

In one procedure, the antigen which is attached to the support is selected to bind the B-cells which produce desired antibody. This procedure is known as an enrichment procedure. Alternatively, the attached antigen can be one which binds undesired antibody. In the latter instance, which is known as a depletion procedure, it is the B-cells in the supernatant and not the bound cells that carry the desired antibody. Thus, if enrichment is being used, it is the bound B-cells which are separated from the plate and then fused with a myeloma cell to produce hybridomas. In depletion, the B-cells in the supernatant are fused to form hybridomas. Irrespective of whether the pre-screening is by depletion or enrichment, the number of B-cells which yield undesired antibody is reduced. As a result, a greater proportion of the hybridomas will produce the desired monoclonal antibodies.

In another embodiment of the invention, the pre-screening is done completely in suspension. In one

mode, undesired antigen carrying some epitopes in common with the desired antigen is added to a suspension of B-cells. Those carrying antibody to this antigen will form aggregates. The supernatant, which contains a greater percentage of the desired B-cells, is used in the fusion to form hybridomas. In another mode, the attempt is to isolate anti-idiotype antibodies in a suspension of cells. This is done by locating aggregates of cells, and then separating these from the suspension. The aggregates should contain B-cells which produce anti-idiotype antibodies.

The invention is described with greater particularity below.

Detailed Description of the Invention

The pre-screening procedure of the invention allows B-cells which produce antibody to selected antigens to be isolated prior to fusion. Thus, following fusion of the pre-screened B-cells with myeloma cells, a greater proportion of the hybridomas will produce desired monoclonal antibodies. The presence of a greater proportion of desired hybridomas makes it easier to locate desired hybridomas and reduces the time normally needed to do so.

The B-cell pre-screening procedure significantly reduces the time needed to locate hybridomas which are specific to an epitope which exists only on a combination of protein subunits. Such an epitope is termed a combinatorial epitope. As discussed above, hybridomas unique to combinatorial epitopes are normally isolated by producing the monoclonal antibodies and then separately testing their reactivity with the subunits and the combinatorial epitope. This is a time-consuming procedure. Pre-screening the B-cells to obtain a

greater proportion of those which produce the antibody to the combinatorial epitope, and then using the pre-screened B-cells in the fusion, produces a greater proportion of desired hybridomas. Consequently, the desired hybridomas can be isolated more easily and more quickly.

An example of using a B-cell pre-screening procedure in the production of monoclonal antibodies unique to combinatorial epitopes is set forth below.

Example I: Selecting Monoclonal Antibodies to a Combinatorial Epitope

Immunization

The combination of the protein subunits ovalbumin and hemocyanin was selected as the combinatorial entity. An ovalbumin-hemocyanin conjugate was prepared by using a one to one molecular ratio of each protein, and adding 25% glutaraldehyde as the cross linker. A more complete description of the cross-linking process is in S. Avrameas, "Coupling of Enzymes to Proteins with Glutaraldehyde," Immunochemistry Vol. 6, 43 (1969), which is incorporated by reference.

After preparation of the conjugate it was used to immunize ten (10) female Balb/c mice. The conjugate was diluted in normal saline (0.9%) and then emulsified in Freunds complete adjuvant by drawing it in and out of a syringe. The final solution contained 100 micrograms of conjugate per 100 microliters. 100 microliters were injected intradermally.

The mice were given booster injections, either subcutaneously or intra-peritoneally, every thirty days. For the boosters, the conjugate was

emulsified in incomplete Freunds adjuvant. At least two booster injections were given to each mouse.

Four to six weeks after the last booster was given, the mice were again immunized with the same amount and concentration of conjugate (mixed in normal saline) by intraspleenic injection. Three to four days later the mice were sacrificed and their spleens were removed in preparation for the pre-screening procedure.

A. <u>Pre-screening Procedure (Depletion)</u>

To increase the proportion of hybridomas which produce antibody specific to the combinatorial epitope, a B-cell pre-screening procedure was carried out using the depletion method.

Ten 175 mm petri dishes were coated with ovalbumin at a concentration of 1 mg/ml in Phosphate Bufferred Saline, and left overnight at 4°C. Excess coating solution was washed off by rinsing with Phosphate Bufferred Saline. 1×10^7 spleen cells were added to each dish and allowed to equilibrate for one hour. During this period the B-cells producing antibody to the ovalbumin attached to the antigen which was bound to the petri dish. Thereafter, the unattached B-cells (which included those B-cells producing antibody to the combinatorial epitope), were recovered by gentle washing and pipetting. These recovered cells, taken from the supernatant, were used in the fusion process.

<u>Fusion Process</u>

Single cell suspensions of the recovered cells were prepared. The recovered cells were diluted in

Dulbeccos Modification of Eagles Basal Medium and spun in a centrifuge at 1000 g for ten minutes. The supernatant was withdrawn, and Sp2/0-M5 myeloma cells (available from the American Tissue Culture Collection in Rockville, Maryland) were added to the cell pellet and resuspended.

The resultant suspension was then spun at 1000 g for ten minutes, and the supernatant was withdrawn. To complete the fusion, 2 ml of polyethylene glycol 4000 was added gradually over ten minutes, together with 20 ml of Dulbeccos Medium.

The fused cells were spun down, the supernatant was withdrawn, and the cells were resuspended in 100 ml of Dulbeccos Medium and added in aliquots of 100 microliters to each of ninety-six wells of a linbro plate. Ten of the ninety-six well plates were so prepared, giving a total of nine hundred and sixty wells available for growth. The fusion products were grown in the plates for two weeks. A similar hybridoma production procedure is described in K. Flurkey, M.B. Bolger & D.S. Linthicum, "Preparation and Characteristics of Antisera and Monoclonal Antibodies to Serotonergic and Dopaminergic Ligands," 8 J. Neuroimmunol. at 115-27 (1985), which is incorporated by reference.

After two weeks the monoclonal antibodies in the wells were checked for reactivity with ovalbumin, hemocyanin, and the ovalbumin-hemocyanin conjugate. The results appear in Table I below.

B. Pre-Screening Procedure (Enrichment)

If the pre-screening was to be done by the enrichment method, essentially the same procedure

described above would be followed. The only difference would be that the conjugate, not the ovalbumin, would be bound to the petri dishes. The B-cells would be passed over the petri dish, as described above. However, B-cells which are specific to the conjugate, not the ovalbumin, would bind to the plate. These bound B-cells would then be separated from the plate and fused with myeloma cells to form hybridomas.

C. Comparison Experiment (Without Pre-screening)

A fundamentally identical procedure to that described in Example I(A) was performed, i.e., the same immunization and fusion process was done. The only difference was that no pre-screening was performed.

Following fusion and hybridoma production, the hybridomas were limit diluted so that there was one clone per well. The monoclonal antibodies in the wells were checked for reactivity with ovalbumin, hemocyanin, and the ovalbumin-hemocyanin conjugate. The results appear in Table I below.

TABLE I

Summary of Changes of Monoclonal Antibodies' Specificities Before and After Pre-Screening

Antigen	Results with Pre-Screening (Number of Reactive Clones for the Listed Antigen)
Ovalbumin	39
Hemocyanin	59
Ovalbumin-Hemocyanin Conjugate	62

Antigen	Results without Pre-Screening (Number of Reactive Clones for the Listed Antigen)
Ovalbumin	183
Hemocyanin	76
Ovalbumin-Hemocyanin Conjugate	36

From Table I it can be seen that where pre-screening was used, a greater percentage of the clones which produced reactive monoclonals produced monoclonals which were specific to the conjugate. In fact, where pre-screening was used 39% of the clones produced monoclonals specific to the conjugate, while only 24% were specific to ovalbumin alone. In contrast, in the procedure where pre-screening was not used, 62% of the clones producing reactive antibody, produced monoclonals which reacted with ovalbumin while only 9% were specific for the conjugate. The value of B-cell

pre-screening in producing clones specific to the combinatorial epitope on the conjugate is apparent.

Related Applications

A B-cell pre-screening procedure would be useful for producing monoclonals to other types of combinatorial epitopes. For example, it could be used in producing monoclonals to the dimer CK-MB.

CK-MB is known to be found in body fluids following myocardial infarction. A monoclonal antibody specific to CK-MB could be useful in developing a test for the presence of myocardial infarction. However, the problem with developing a monoclonal to CK-MB is that the proteins CK-MM and CK-BB, both of which are normally present in body fluids, also carry the CK subunit. Therefore, immunization with CK-MB will yield antibodies which react with CK-MM, CK-BB and with CK-MB. A B-cell pre-screening procedure could be used in the manner set forth below to select B-cells which produce antibody specific only to CK-MB, which will not bind with CK-MM or CK-BB.

Example II: Producing Monoclonal Antibodies Specific to CK-MB and Other Dimers

Essentially the same procedure described in Example I is used to produce monoclonal antibodies to the heart muscle enzyme CK-MB. The major difference is that the immunization is done with CK-MB instead of the ovalbumin-hemocyanin conjugate. The other main difference is that the pre-screening procedure is done with CK-MM and CK-BB bound to the solid support. As a result, B-cells which produce antibody to the CK subunit bind to the support. The supernatant, which

contains B-cells specific to CK-MB, is then used in the fusion process. A high percentage of the hybridomas should produce monoclonal antibodies specific to CK-MB.

Related Techniques

Essentially the same procedure as described directly above could also be used to produce monoclonal antibodies to other dimers, for example, to lactate dehydrogenase, and other isoenzymes.

Immunogens with a High Percentage of Commonality

Pre-screening also reduces production time where different immunogenic entities carry most of the same epitopes, and the objective is to produce monoclonal antibodies to the epitopes which are not common to all entities. An example of this is where antibodies are desired which can differentiate a cancerous from a normal cell. The monoclonal antibodies produced could be used in the diagnosis of the disease conditions. An example of using a pre-screening procedure in the selection of monoclonals to cancerous cells is set forth below.

Example III: Producing Monoclonals Specific to Cancer Cells

Using the immunization technique of Example I, host animals are immunized with cancerous cells. The resultant family of B-cells are then pre-screened with normal healthy cells, which have been bound to a solid support. The B-cells which produce antibody to epitopes on the normal cells will bind. The supernatant, which contains B-cells producing antibody specific to cancer cell epitopes, is then collected. A greater proportion

of the monoclonals produced from the fusion of the supernatant B-cells should be specific to the cancerous cells.

Related Applications

Essentially the same B-cell pre-screening procedures described above can be used to produce monoclonal antibodies which differentiate between other types of closely-related cells. However, the immunizing antigen and the bound antigen used would, of course, be different. Similar pre-screening procedures could be used, for example, to produce monoclonal antibodies which distinguish an AIDS transformed T-cell from a normal T-cell, a virus transformed cell from a normal untransformed cell, a young cell from a quiescent cell from a senescent cell, a starved from a non-starved cancer cell, or a transformed cell causing arterio-sclerosis from normal epithelial cells which line the arteries.

Trace Proteins

Pre-screening the B-cells is also valuable where one is attempting to produce monoclonal antibodies to trace proteins, for example, those proteins which are present only in a diseased individual. Monoclonals to these proteins could be used in diagnosis of disease states.

Example IV: Production of Monoclonal Antibodies Specific to Trace Related Proteins

Using the immunization technique of Example I, animals are immunized with whole serum taken from a healthy individual. Other animals are immunized with whole serum from a diseased individual. All serum proteins from the healthy individual are then

attached to a petri dish. B-cells from animals immunized with unhealthy serum are passed over the petri dish. Those B-cells that do not attach to the bound proteins, i.e., those which carry antibody to the diseased proteins, are fused to myeloma cells to produce hybridomas. The resulting monoclonal antibodies are likely to be specific and bind only to disease-induced, or related, proteins.

This method may also be used in the opposite mode, in which monoclonals are produced to proteins that are only present in healthy individuals and absent in diseased individuals.

Related Procedures

B-cell pre-screening would also be useful in producing monoclonal antibodies to trace proteins, for example, those which are present on different strains of viruses. The monoclonal antibodies produced can be used to isolate quantities of the trace proteins for study and analysis.

A related technique to that described directly above can be used to produce monoclonals which differentiate between other closely-related proteins. The bound antigen used in the pre-screening would be a protein which is closely related to the immunizing protein. By choosing appropriate immunizing and bound antigens, antibodies can be produced which differentiate among fully folded, slightly folded and unfolded proteins.

Pre-screening in Suspension

A different type of pre-screening, done completely in suspension, can also be performed. If, for example, monoclonal antibodies specific to CK-MB were to be produced, the following procedure would be used.

Example V: Pre-screening in Suspension

A host animal is immunized with CK-MB, and its spleen is removed. The spleen is ground and suspended. CK-MM is added to the suspension. The aggregates formed by the B-cells and CK-MM are allowed to settle, and the supernatant is withdrawn. CK-BB is then added to the supernatant, the aggregates are again allowed to settle, and the supernatant is withdrawn. The final supernatant should contain a higher proportion of B-cells which produce antibody specific to CK-MB.

Related Procedure

Pre-screening, in suspension is also useful in isolating anti-idiotype antibodies. B-cells which produce anti-idiotype antibody will carry it on their surface and will tend to aggregate with B-cells which produce the idiotype. The occurrence of aggregates in a suspension of B-cells suggests the presence of anti-idiotype antibodies.

B-cell aggregates are relatively easy to isolate because they are heavier than the other components in the suspension and tend to settle. Settled aggregates can be easily collected and examined for the presence of anti-idiotypes. If the aggregates do not settle freely, the suspension can be centrifuged and the aggregates collected. The B-cells which produce the anti-idiotype antibody can then be separated from the other B-cells in the aggregate, and used to produce monoclonals.

It should be understood that the examples herein are intended to be exemplary only and not limiting, and that the scope of protection is defined in the claims which follow, and includes all equivalents of the subject matter of the claims.

What Is Claimed Is:

1. A pre-screening procedure for either selecting or eliminating certain B-cells in a family of B-cells, said certain B-cells being those which produce antibodies to a specific epitope, comprising:

placing a said family of B-cells near enough to said specific epitope so that said certain B-cells can bind thereto.

2. The pre-screening procedure of claim 1 wherein said specific epitope is carried on an immunizing antigen with which a host animal was immunized, and the claim 1 procedure further includes the steps of:

separating bound B-cells from said immunizing antigen; and

fusing said separated B-cells with myeloma cells to form hybridomas.

3. The pre-screening procedure of claim 1 wherein said specific epitope is carried on an immunizing antigen with which a host animal was immunized, and the claim 1 procedure further includes the steps of:

collecting the unbound B-cells; and

fusing said unbound B-cells with myeloma cells to form hybridomas.

4. The pre-screening elimination procedure of claim 1 wherein said specific epitope is carried on an immunizing antigen with which a host animal was immunized, and is also carried on a second antigen, and wherein the family of B-cells is placed near enough to bind to said second antigen.

5. The pre-screening procedure of claim 1 wherein said specific antigen is bound to a solid support.

6. A pre-screening procedure for either selecting or eliminating certain B-cells in a family of B-cells, said certain B-cells being that which produce antibodies to a specific epitope, comprising:

binding an antigen carrying said specific epitope to a solid support;

placing a family of B-cells near enough to said support so that said certain B-cells can bind to said specific epitope; and

collecting either the supernatant containing unbound B-cells or collecting the bound B-cells and using the collected cells for fusion with myeloma cells to produce hybridomas.

7. The pre-screening procedure of claim 6 wherein said specific epitope is carried on an immunizing antigen with which a host animal was immunized, and wherein it is the bound B-cells which are collected.

8. The pre-screening procedure of claim 6 wherein said specific epitope is carried on an immunizing antigen with which a host animal was immunized, and wherein it is the supernatant which is collected.

9. The pre-screening procedure of claim 6 wherein said specific epitope is carried on an immunizing antigen with which a host animal was immunized, and it is also carried on a second antigen, and wherein the second antigen is bound to the solid support, and wherein the supernatant is collected.

10. The pre-screening procedure of claim 9 wherein said immunizing antigen is a conjugate of two or more protein subunits, said specific epitope is carried on one of said protein subunits, and wherein the supernatant is collected.

11. The pre-screening procedure of claim 8 wherein said immunizing antigen is a cancerous cell, a derivative of a cancerous cell, a virally transformed or otherwise abnormal cell, said specific epitope is carried on a normal cell, and wherein the supernatant is collected.

12. The pre-screening procedure of claim 7 wherein said immunizing antigen is a cancerous cell, a derivative of a cancerous cell, a virally transformed or otherwise abnormal cell, said specific epitope is carried on a cancerous cell, a derivative of a cancerous cell, a virally transformed or otherwise abnormal cell which is bound to the solid support, and wherein the bound B-cells are collected.

13. A pre-screening procedure for eliminating B-cells which produce antibodies to various epitopes of protein but not to a specific epitope, comprising:

immunizing a host animal with a dimer having two separate protein subunits and one distinct epitope;

coating at least one of said protein subunits on a solid support;

passing a family of B-cells from the host animal over said support so as to bind to the support those B-cells which produce antibody specific to said bound protein subunit;

collecting the B-cells in the supernatant; and

fusing the collected B-cells with myeloma cells to produce hybridomas.

14. The pre-screening procedure of claim 13 wherein the dimer is ovalbumin-hemocyanin and the protein subunit bound to the solid support is ovalbumin.

15. The pre-screening procedure of claim 13 wherein the dimer is the heart muscle protein CK-MB, and the protein subunit bound to the solid support is CK-BB and/or CK-MM.

16. The pre-screening procedure of claim 13 wherein the dimer is lactate dehydrogenase, or another isoenzyme.

17. A pre-screening procedure for eliminating B-cells which do not produce antibodies to epitopes on a trace protein, comprising:

immunizing a first host animal with whole serum from a healthy individual, and immunizing a second host animal with whole serum from a diseased individual;

attaching serum proteins from the first host animal to a solid support;

passing a family of B-cells from said second host animal over the solid support; and

collecting the unreactive B-cells in the supernatant and fusing them with myeloma cells to form hybridomas.

18. A pre-screening procedure for eliminating B-cells which produce antibodies to structurally related proteins comprising:

immunizing a host animal with a first protein;

attaching a second protein which is structurally related to said first protein to a solid support;

passing a family of B-cells from said host animal over said solid support; and

collecting the unreactive B-cells in the supernatant and fusing them with myeloma cells to form hybridomas.

19. The pre-screening process of claim 18 wherein the first protein is a virus and the second protein is a different strain of virus.

20. The pre-screening process of claim 18 wherein the first protein is a young cell and the second protein is a quiescent or senescent cell.

21. A pre-screening process for eliminating B-cells which do not produce antibodies to a trace protein, comprising:

immunizing a host animal with serum which does not contain the trace protein;

withdrawing serum from the animal and attaching the serum protein to a solid support;

passing a family of B-cells from a second host animal, which has been immunized with serum which does contain the trace protein, over said solid support; and

collecting the unreactive B-cells in the supernatant and fusing them with myeloma cells to form hybridomas.

22. A pre-screening process for selecting B-cells which produce anti-idiotype antibody, comprising:

locating aggregates of B-cells within a suspension of a family of B-cells; and

separating the aggregates and examining the B-cells to determine which produce anti-idiotype antibody.

23. The pre-screening process of claim 22 further including the steps of isolating the anti-idotype producing B-cells and fusing them with myeloma cells to form hybridomas.

24. A pre-screening process for selecting B-cells which produce antibodies to a specific epitope, comprising:

immunizing a host animal with a first antigen which carries said specific epitope;

withdrawing B-cells from the host animal and suspending the B-cells;

adding to the suspension at least one antigen which has at least one epitope in common in the said first antigen, but where the common epitope is not said specific epitope;

collecting the unreactive B-cells in the supernatant and fusing them with myeloma cells to form hybridomas.

25. The pre-screening process of claim 24 wherein the specific epitope is carried by the protein CK-MB, and the antigens CK-MM and CK-BB are added to the suspension.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/02407

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): C12Q 1/70; G01N 33/53, 33/543, 33/554, 33/538

U.S. CL: 435/5,7; 436/518, 519, 541, 548, 824; 935/47, 90, 92

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
U.S.	435/5,7; 436/518, 519, 541, 548, 824; 935/47, 90, 92

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X Y	F.C. Hay, "Idiotype Vaccines", in Immune Intervention, published 1984 by Academic Press (London), see pages 117-138.	1,2, 4-7 3, 8-25
Y	Pharmacia Fine Chemicals technical disclosure, printed August 1982 by Ljungforetagen AB (Sweden), "Affinity Chromatography Principles and Methods" pages 96-97.	3, 8-11, 13-25
Y	E.D. Sevier et al, "Monoclonal Anti-bodies in Clinical Immunology" in Clinical Chemistry, 1981, 27(11), 1797-1806.	12-16, 19

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
7 November 1988	03 FEB 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Eric Vacchio Eric Vacchio

Form PCT/ISA/210 (second sheet) (Rev.11-87)

International Application No.
PCT/US88/02407

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,Y	US, A, 4,746,539 (The Ohio State University Research Foundation) 24 May 1988, see column 8 lines 32-40.	11,17, 21
Y	US, A, 3,932,221 (Merck Patent Gesellschaft mit beschrancter Haftung) 13 January 1976, see columns 4-5	15,16, 25
Y	B. Alberts et al, "Molecular Biology of the Cell" published 1983 by Garland Publishing, Inc. (New York), see pages 928-929.	20
Y	H.N. Eisen, "Immunology" published 1980 by Harper and Row, Publishers (Philadelphia), see page 394.	22-25
Y	D.P. Stites et al, "Basic and Clinical Immunology" published 1982 by Lange Medical Publications (Los Altos, California), see pages 165 and 373.	22-25